



ANNOUNCEMENT

Interested Members of the University community are invited to attend the Final Oral Examination for the degree of **Doctor of Philosophy** of

Ari Mendell

of the Department of Biomedical Sciences (Ontario Veterinary College) on Tuesday, December 18th at 1:00pm in the Lifetime Learning Centre Room 1713 (Seminar); and PAHL Room 3826 (Examination)

The Role of Neurosteroid Metabolites of Gonadal Steroid Hormones in Neuroprotection

Examination Committee

Dr. Neil MacLusky, Advisor
Dr. Boyer Winters, Committee Member
Dr. Jane Rylett, External Examiner
Dr. Jibran Khokhar, Graduate Faculty
Dr. Tarek Saleh, Exam Chair

Advisory Committee

Dr. Neil MacLusky
Dr. Boyer Winters
Dr. Bettina Kalisch
Dr. Jonathan LaMarre

ABSTRACT

Gonadal steroid hormones are known to protect the brain against the development of neurodegenerative conditions, especially Alzheimer's disease (AD). Women are approximately twice as likely to develop AD compared to men, which has been attributed to the drastic and abrupt decline of circulating ovarian steroid hormone levels that occurs around the time of menopause. Testosterone levels decline more gradually in aging men, which may account for the relative protection of the male brain. However, even the gradual age-related decline in circulating testosterone levels is associated with an increased risk for development of AD. Recent evidence has highlighted previously underappreciated protective roles of gonadal steroid metabolites that are synthesized in the brain. One such neurosteroid – 5 α -androstane-3 α ,17 β -diol (3 α -diol) – is a metabolite of testosterone that may contribute to the effects of its precursor by acting through distinct cellular mechanisms. However, possible functions of 3 α -diol in preventing neurotoxicity remain largely uncharacterized. In this thesis, the neuroprotective effects of 3 α -diol and related neurosteroids are explored, with an emphasis on their potential role in the prevention of AD-related pathology.

Study 1 demonstrates that physiological concentrations of 3 α -diol protect neurons against oxidative stress and AD-related toxicity in the form of amyloid β (A β) exposure *in vitro*, by inhibiting dysregulated ERK signaling, caspase-3 activation, and cell death. Study 2 demonstrates that 3 α -diol and the progesterone-derived analog, allopregnanolone, differentially inhibit ERK phosphorylation induced by A β in the presence and absence of neurosteroid-sensitive GABA_A receptors, suggesting an alternative mechanism of action for 3 α -diol. Study 3 demonstrates that the protective effects of 3 α -diol against dysregulated ERK phosphorylation and associated neurotoxicity are dependent on modulation of the ERK-directed phosphatase, MKP3/DUSP6. Finally, study 4 demonstrates that inhibiting the synthesis of testosterone-derived neurosteroids in male 3xTg-AD mice impairs object recognition memory, dysregulates dendritic morphology, and exacerbates AD-related pathology and signaling dysfunction in the hippocampus. Collectively, the findings presented in this thesis characterize the neuroprotective effects of 3 α -diol using both *in vitro* and *in vivo* models of AD, and suggest that this neurosteroid may play an important role in preventing the development of neurodegenerative disease.

PUBLICATIONS

Mendell, A.L. and MacLusky, N.J. (2018) Neurosteroid metabolites of gonadal steroid hormones in neuroprotection: implications for sex differences in neurodegenerative disease. *Frontiers in Molecular Neuroscience* 11:359.

Mendell, A.L., Chung, B.Y., Creighton, C.E., Kalisch, B.E., Bailey, C.D., MacLusky, N.J. (2018) Neurosteroid metabolites of testosterone and progesterone differentially inhibit ERK phosphorylation induced by amyloid β in SH-SY5Y cells and primary cortical neurons. *Brain Research* 1686: 83-93.

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Louth, E.L., Sutton, C.D., **Mendell, A.L.**, MacLusky, N.J., Bailey, C.D.C. (2017) Imaging neurons within thick brain sections using the Golgi-Cox method. *Journal of Visualized Experiments* 122.

Mendell, A.L., Atwi, S., Bailey C.D., McCloskey D., Scharfman, H.E., MacLusky, N.J. (2017) Expansion of mossy fibers and CA3 apical dendritic length accompanies the fall in dendritic spine density after gonadectomy in male, but not female, rats. *Brain Structure and Function* 222(1): 587-601.

Penney, J., **Mendell, A.L.**, Zeng, M., Tran, K., Lymer, J., Turner, P.V., Choleris, E., MacLusky, N., Lu, R. (2017) LUMAN/CREB3 is a key regulator of glucocorticoid-mediated stress responses. *Molecular and Cellular Endocrinology* 439: 95-104.

Mendell, A.L., Creighton, C.E., Kalisch, B.E., MacLusky, N.J. (2016) 5α -androstane- $3\alpha,17\beta$ -diol inhibits neurotoxicity in SH-SY5Y human neuroblastoma cells and mouse primary cortical neurons. *Endocrinology* 157(12): 4570-4578.

Mendell, A.L., Szigeti-Buck, K., MacLusky, N.J., & Leranth, C. (2014). Orchidectomy does not significantly affect spine synapse density in the CA3 hippocampal subfield in St. Kitts vervet monkeys (*Chlorocebus aethiops sabaenus*). *Neuroscience Letters* 559: 189-192.

Mendell, A.L., MacLusky, N.J., & Leranth, C. (2013). Unilateral fimbria/fornix transection prevents the synaptoplastic effect of dehydroepiandrosterone in the hippocampus of female, but not male, rats. *Neuroscience & Medicine* 4(3): 134-139.

Mendell, A.L., MacLusky, N.J. The testosterone metabolite 3α -androstenediol inhibits oxidative stress-induced ERK phosphorylation and neurotoxicity in SH-SY5Y cells through a MKP3/DUSP6-dependent mechanism. *Neuroscience Letters* (submitted manuscript – under review).

Creighton, S.D., **Mendell, A.L.**, Palmer, D., Kalisch, B.E., MacLusky, N.J., Prado, V.F., Prado, M.A.M., Winters, B.D. Beyond “object recognition” in transgenic mouse models of Alzheimer’s disease: dissociable disturbances in specific aspects of object processing in male and female 5xFAD and 3xTG mice. *Scientific Reports* (submitted manuscript – under review).

Mitchnick, K.A., **Mendell, A.L.**, Wideman, C.E., Muller, A-M., Creighton, S.D, Choleris, E.C., MacLusky, N.J., Winters, B.D. Estrogen and estrogen receptor involvement in perirhinal cortex-mediated object memory. *Psychoneuroendocrinology* (submitted manuscript – under review).

Mendell, A.L., Creighton, S.D., Wilson, H.A., Isaacs, L., Winters, B.D., MacLusky, N.J. Finasteride impairs memory, dysregulates hippocampal dendritic morphology, and increases pathology in male 3xTg-AD mice. *Manuscript in preparation*.

Wilson, H.*, **Mendell, A.L.***, MacLusky, N.J., Hunter, R. G. Sex, stress and the developing brain.

*The two indicated authors contributed to the manuscript equally. *Manuscript in preparation.*

SELECTED CONFERENCE PRESENTATIONS

(Partial list – total of 32 presentations as presenting or contributing author)

Poster presentation at the 2018 Society for Neuroscience annual meeting (International). Presenting author.

Poster presentation at the 2018 International Congress of Neuroendocrinology meeting (International). Presenting author.

Oral presentation at the 2018 OVC Graduate Student Research Symposium (Local). Presenting author.

Poster presentation at the 2018 Canadian Association for Neuroscience annual meeting (National). Presenting author.

Poster presentation at the 2018 Southern Ontario Neuroscience Association annual meeting (Provincial). Presenting author.

Poster presentation at the 2017 Organization for the Study of Sex Differences annual meeting (International). Presenting author.

Oral presentation at the 2017 University of Guelph Neuroscience Day (Local). Presenting author.

Poster presentation at the 2016 Society for Neuroscience annual meeting (International). Presenting author.

Poster presentation at the 2016 Canadian Association for Neuroscience annual meeting (National). Presenting author.

Poster presentation at the 2016 Organization for the Study of Sex Differences annual meeting (International). Presenting author.

Poster presentation at the 2016 Southern Ontario Neuroscience Association annual meeting (Provincial). Presenting author.

Poster presentation at the 2016 Endocrine Society annual meeting (International). Presenting author.

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BIOGRAPHICAL DATA

Ari Mendell graduated from the University of Guelph with an Honours Bachelor of Science degree, majoring in Biological Sciences and minoring in Neuroscience, in 2012. His interest in neuroscience began early in his undergraduate studies, and expanded during his fourth year undergraduate research project with Dr. Neil MacLusky. He started his thesis-based M.Sc degree in 2012, focusing on the effects of androgens on hippocampal synaptic plasticity and dendritic morphology, and defended his thesis in 2014. He then began the Ph.D program in 2015, studying the role of neurosteroid metabolites of gonadal steroid hormones in neuroprotection. He is particularly interested in how neurosteroid synthesis may contribute to the sex differences in the development of Alzheimer's disease.

AWARDS RECEIVED

NSERC Alexander Graham Bell Canada Graduate Scholarship – Doctoral CGS-D (2016-2019)

Trainee Professional Development Award for Neuroscience 2018 – Society for Neuroscience (2018)

First Place – Ph.D Oral Presentation – Ontario Veterinary College Graduate Research Symposium (2018)

First Place – Ph.D Poster Presentation – Southern Ontario Neuroscience Association (2018)

Travel Award for CAN annual meeting – Canadian Association for Neuroscience (2018)

Biomedical Sciences Graduate Scholarship – Ontario Veterinary College (2014, 2016, 2017, 2018)

Ontario Veterinary College Doctoral Scholarship (2015-2018)

Best Oral Presentation Award – University of Guelph Neuroscience Day (2017)

Ontario Graduate Scholarship (held 2013-2014; declined in favour of NSERC CGS-D in 2016)

Harry G. Downie Travel Grant – Ontario Veterinary College (2016)

Barbara Kell Gonsalves Memorial Scholarship – Ontario Veterinary College (2014)

Pari S. Basrur Travel Scholarship – Ontario Veterinary College (2014)

First place – M.Sc Poster Presentation (co-recipient) – Southern Ontario Neuroscience Association (2013)

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